Pest Management Regulatory Agency
Agence de réglementation de la lutte antiparasitaire

Proposed Regulatory Decision Document PRDD2007-01

RootShield Biological Fungicide
Trichoderma harzianum Rifai strain KRL-AG2

RootShield Technical Biological Fungicide and associated end-use products RootShield Drench Biological Fungicide Wettable Powder and RootShield Granules Biological Fungicide, containing the fungal microorganism *Trichoderma harzianum* Rifai strain KRL-AG2, are proposed for full registration under the Pest Control Products Regulations for the suppression of soilborne diseases in greenhouse crops (tomatoes, cucumbers and ornamentals).

This Proposed Regulatory Decision Document provides a summary of data received and the rationale for the proposed full registration of these products. Health Canada's Pest Management Regulatory Agency (PMRA) will accept written comments on this proposal up to 45 days from the date of publication of this document. Please forward all comments to Publications at the address below.

(publié aussi en français)

9 January 2007

This document is published by the Alternative Strategies and Regulatory Affairs Division, Pest Management Regulatory Agency. For further information, please contact:

Publications
Pest Management Regulatory Agency
Health Canada
2720 Riverside Drive
A.L. 6605C
Ottawa, Ontario
K1A 0K9

Internet: pmra_publications@hc-sc.gc.ca
www.pmra-arla.gc.ca
Information Service:
1-800-267-6315 or 613-736-3799
Facsimile: 613-736-3758

Health Canada Santé Canada

Canada

ISBN: 978-0-662-44806-8 (978-0-662-44807-5)
Catalogue number: H113-9/2007-1E (H113-9/2007-1E-PDF)

© Her Majesty the Queen in Right of Canada, represented by the Minister of Public Works and Government Services Canada 2007

All rights reserved. No part of this information (publication or product) may be reproduced or transmitted in any form or by any means, electronic, mechanical photocopying, recording or otherwise, or stored in a retrieval system, without prior written permission of the Minister of Public Works and Government Services Canada, Ottawa, Ontario K1A 0S5.

Foreword

The submissions for full registration of *Trichoderma harzianum* Rifai strain KRL-AG2 in RootShield Technical Biological Fungicide and its end-use products RootShield Drench Biological Fungicide Wettable Powder and RootShield Granules Biological Fungicide, manufactured by BioWorks Inc., for the suppression of root pathogens in greenhouse tomatoes, cucumbers and ornamentals have been reviewed by Health Canada's Pest Management Regulatory Agency. These products were submitted under the User Requested Minor Use Registration Program with support from the National Greenhouse Vegetable Minor Use Committee.

The PMRA had previously issued a temporary registration for these products, published in Regulatory Note REG2002-01, *Rootshield Biological Fungicide* Trichoderma harzianum *Rifai strain KRL-AG2*, with the requirement that BioWorks Inc. carry out additional studies. These studies have now been completed.

RootShield Drench Biological Fungicide Wettable Powder and RootShield Granules Biological Fungicide are microbial biopesticide products containing 1.15% *T. harzianum* Rifai strain KRL-AG2, a modified strain of a naturally occurring soil fungus. RootShield end-use products are proposed for suppression of root pathogens in greenhouse crops. These RootShield products will be especially valuable in the production of greenhouse tomatoes and cucumbers; presently, there are few disease control products for these crops and non-chemical options are preferred. Microbial biopesticides are increasingly being investigated for use as alternatives to conventional chemical pesticides because they are thought to pose a lower potential risk to human health and the environment. The RootShield wettable powder and granular formulations represent reduced-risk options to chemical fungicide management tools.

The PMRA has carried out an assessment of available information in accordance with the Pest Control Products Regulations and has found it sufficient to allow a determination of the safety, merit and value of the technical grade active ingredient RootShield Technical Biological Fungicide and end-use products RootShield Drench Biological Fungicide Wettable Powder and RootShield Granules Biological Fungicide. The Agency has concluded that the use of the microorganism *T. harzianum* Rifai strain KRL-AG2 in the technical grade active ingredient RootShield Technical Biological Fungicide and RootShield end-use products in accordance with the label has merit and value consistent pursuant to the Pest Control Products Regulations and does not entail an unacceptable risk of harm. Therefore, based on the considerations outlined above, the use of the microbial *T. harzianum* Rifai strain KRL-AG2 and RootShield end-use products for suppression of root pathogens in greenhouse tomatoes, cucumbers and ornamentals are proposed for full registration pursuant to the Pest Control Products Regulations.

Table of Contents

1.0 The Active Ingredient, Its Properties and Uses 1
1.1 Identity of the Active Ingredient and Impurities 1
1.2 Physical and Chemical Properties of the Technical Grade Active Ingredient and the End-Use Products 2
1.3 Details of Uses and Further Information 2

2.0 Methods of Analysis 3
2.1 Methods for Analysis of the Microorganism as Manufactured 3
2.1.1 Methods for Identification of the Microorganism 3
2.1.2 Methods for Establishment of Purity of Seed Stock 4
2.1.3 Methods to Define the Content of the Microorganism in the Manufactured Material Used for the Production of Formulated Products . 5
2.1.4 Methods for the Determination of Relevant Impurities in the Manufactured Material 5
2.1.5 Methods for Demonstration of Absence of Any Human and Mammalian Pathogens 7
2.1.6 Methods for Determination of Storage Stability, Shelf-Life of the Microorganism 7
2.2 Methods for Determination and Quantification of Residues (viable or non-viable) of the Active Microorganism and Relevant Metabolites 7

3.0 Impact on Human and Animal Health 8
3.1 Integrated Toxicity and Infectivity Summary 8
3.2 Reporting of Hypersensitivity Incidence 9
3.3 Impact on Human and Animal Health Arising from Exposure to the Active Substance or to Impurities Contained in It 9
3.3.1 Occupational and Bystander Exposure Assessment 9

4.0 Residues 10
4.1 Residue Summary 10
4.2 Maximum Residue Limit 11

5.0 Fate and Behaviour in the Environment 12
5.1 Summary of Fate and Behaviour in the Terrestrial Environment 12
5.1.1 Field Studies 12
5.1.2 Conclusions 12

6.0 Effects on Non-Target Species 12
6.1 Effects on Terrestrial Organisms 13
6.2 Effects on Aquatic Organisms 13
6.3 Risk Characterization 13

7.0 Efficacy 14
7.1 Effectiveness on Selected Diseases 14
7.2 Phytotoxicity/Pathogenicity to Target Plants or to Target Plant Products 16
7.3 Compatibility with Current Management Practices Including Integrated Pest Management 16
7.4 Contribution to Risk Reduction 16
7.5 Information on the Occurrence or Possible Occurrence of the Development of Resistance 16
7.6 Conclusions 17

8.0 Toxic Substances Management Policy Considerations 17

9.0 Proposed Regulatory Decision 17

List of Abbreviations 19

Appendix I Summary Tables 21
Table 1 Summary of Toxicity and Pathogenicity Studies with RootShield Biological Fungicide (*Trichoderma harzianum* strain KRL-AG2) 21
Table 2 Summary of Effects on Terrestrial Organisms 23
Table 3 Summary of Effects on Aquatic Organisms 26

1.0 The Active Ingredient, Its Properties and Uses

1.1 Identity of the Active Ingredient and Impurities

Active microorganism	*Trichoderma harzianum* strain KRL-AG2
Function	Biological fungicide
Binomial name	*Trichoderma harzianum* Rifai strain KRL-AG2
Taxonomic designation	
Kingdom	Fungi
Phylum	Deuteromycotina
Order	Hyphomycetes (syn. Moniliales)
Genus	*Trichoderma*
Species	*harzianum*
Strain	KRL-AG2
Canadian patent status information	None
Nominal purity of active ingredient	100% of the "technical" preparation is the active ingredient corresponding to a minimum of 5.0×10^8 colony forming units (CFU)/g dry weight of *T. harzianum* strain KRL-AG2. RootShield end-use products contain 1.15% w/w (equivalent to a minimum 10^7 CFU/g).
Identity of relevant impurities of toxicological, environmental and/or other significance	The technical product does not contain any impurities or microcontaminants known to be Toxic Substances Management Policy (TSMP) Track 1 substances. A bacterial load of $> 10^7$ CFU/g or the presence of a human pathogen will result in the rejection of a starter culture, termination of the production process or discarding of the final product. A stock culture is rejected if any fungal or bacterial contamination is found to be present. RootShield end-use products may contain antibiotic peptides collectively known as peptaibols. The absence of toxic effects in mammalian acute toxicity studies (see Section 3.0) suggests that the manufacturing process either does not favour the production of these potentially toxic metabolites or that the levels produced are too low to elicit an effect in animals administered a high dose of this fungus.

1.2 Physical and Chemical Properties of the Technical Grade Active Ingredient and the End-Use Products

Technical Grade Active Ingredient—RootShield Technical Biological Fungicide
Not required. RootShield Drench Biological Fungicide Wettable Powder (RootShield Drench WP) and RootShield Granules Biological Fungicide (RootShield Granules) end-use products are manufactured following a continuous manufacturing process that does not involve an intermediate stand-alone technical product.

End-Use Products—RootShield Granules and RootShield Drench WP

Property	RootShield Granules	RootShield Drench WP
Physical state at 25°C	Coarse granular powder	Fine granular powder
Colour	Grey or green Standard Munsell 7.5Y 5/1.4	Grey or green Standard Munsell 2.5Y 8.3/2
Odour	Odourless to earthy	Odourless to earthy
pH in distilled water	7.05	7.92
Density	Bulk density: 0.61 g/cm^3 Tap density: 0.68 g/cm^3	Bulk density: 0.29 g/cm^3 Tap density: 0.63 g/cm^3
Viscosity	Not applicable	Not applicable
Corrosion character	No evaluation performed	No evaluation performed
Suspendibility	No evaluation performed	No evaluation performed
Moisture content	No evaluation performed	No evaluation performed

1.3 Details of Uses and Further Information

RootShield Drench WP and RootShield Granules are both proposed for suppression of root diseases caused by *Pythium*, *Rhizoctonia* and *Fusarium* in greenhouse food crops (Use-site Category #5) and non-food crops (Use-site Category #6). The products are to be applied to growing medium for tomatoes, cucumbers and ornamental plants and directly to ornamental bulbs. The wettable powder formulation is applied as a drench by combining product with sufficient water to achieve uniform application and spraying (low pressure) onto planting mix. Rates of application vary from 6 to 15 g product per square metre of cultivated area or 150 g product per 1000 pots (5–8 cm in diameter). Ornamental bulbs may be dipped in a suspension of 120 g/L prior to planting. The granular formulation is added to potting soil at rate of 750 g product per cubic metre (m^3) of soil by raking or tilling into soil in planting beds or by incorporating during potting mix preparation.

Trichoderma is a genus of filamentous deuteromycetes that is ubiquitous in the environment. Its members are generally found in all soils including forest humus layer as well as in agricultural and orchard soils. *Trichoderma* species are rarely reported to occur on living plants and have not been found as endophytes of living plants. *Trichoderma harzianum* is an antagonist of soilborne fungal pathogens. Its mode of action is complex, involving chemotaxis, antibiosis and parasitism. The initial interaction between the parasite and its host appears to be chemotropic growth. The hypha of the mycoparasite grows directly towards its host in response to secreted lectin(s). Apparently, lectins produced by the host bind to the galactose residues on *T. harzianum*'s cell wall and allow it to locate its prey. The antagonistic process starts in advance of physical contact. *Trichoderma harzianum* secretes a number of cell wall degrading enzymes and antibiotics. These cell wall degrading enzymes include β-1,3-glucanases, chitinases and proteinases. Several different volatile and non-volatile antibiotics—such as diterpenes, peptaibols, butenolides, furanones, pyrones and pyridones—have been reported for *T. harzianum*. It is believed that these enzymes and antibiotics provide a synergistic effect on its host. It appears that the weakening of the host cell wall by the enzymes increases the rate of diffusion of the antibiotics through the cell wall. Upon physical contact, the hyphae of *T. harzianum* coil around its host where they proceed with invasive growth. Shortly afterwards, the host hyphae collapse due to a loss of turgor pressure.

Trichoderma harzianum strain KRL-AG2 was derived from the fusion of two auxotrophic strains of *T. harzianum*, strain T12m-2 his^- and strain T95-1 lys^-. Strain T12m-2 his^- is a histidine-deficient auxotroph derived from strain T12m by ultraviolet (UV) light mutation. T12m is a spontaneous derivative of strain T12 (a natural soil isolate from Geneva, New York) obtained by selection without mutation treatment for resistance to cycloheximide as a strain maker. Strain T95-1 lys^- is a lysine-deficient auxotroph derived by UV light mutation and selection from strain T95, a benomyl-resistant mutant derived from a naturally occurring strain isolated from Colombia, South America.

Strain T12 and T95 were selected for protoplast fusion because of their ability to control several plant pathogens. Strain T95 is also capable of colonizing roots of plants grown from inoculated seeds. Strain KRL-AG2 is derived primarily from strain T12. Molecular markers, i.e., four isozyme loci for which the parental strains express different alleles, are of the T12 phenotype. Strain KRL-AG2 is also sensitive to benomyl, as is T12, but not T95. Although both the derivatives of T12 and T95 used for protoplast fusion were auxotrophic, KRL-AG2 is completely prototrophic.

2.0 Methods of Analysis

2.1 Methods for Analysis of the Microorganism as Manufactured

2.1.1 Methods for Identification of the Microorganism

The applicant detailed appropriate methodologies for detection, isolation and enumeration of the active ingredient, strain KRL-AG2. The microbial pest control agent (MPCA) is identified using two different techniques: isozyme analysis, which is used to evaluate starter cultures prior to use

in production, and selective plating on growth media to distinguish between strain KRL-AG2 and other *Trichoderma* strains based on colony morphological traits. Vegetative growth is in the form of hyphae, and asexual reproduction occurs via the production of conidiospores and chlamydospores. Sporulation is influenced by such factors as nutrition, pH and light.

The identification of *T. harzianum* to the species level is achieved using standard mycological techniques for this genus. Additional tests to distinguish strain KRL-AG2 from other *Trichoderma* species and strains of *T. harzianum* included isozyme electrophoresis and colony morphology on differential growth medium developed for strain recognition. The taxonomic position of strain KRL-AG2 was confirmed by microscopic examination of asexual reproductive structures (conidiospores and phialides) according to Rifai's species description.

Using starch gel electrophoresis, strain KRL-AG2 is differentiated from most other strains of *Trichoderma* by comparing 17 isozyme patterns to known allelic profiles. Known exceptions are *T. harzianum* strain T12 (and derivatives) and strain 1892, which express the same allele patterns as KRL-AG2 at the isozyme loci tested. An auxotrophic mutant of strain T12 was used in the production of strain KRL-AG2 by protoplast fusion techniques.

Trichoderma harzianum strain KRL-AG2 is also distinguished from other strains including strain T12 by colony morphology when propagated on CCNS differential medium. CCNS medium is comprised of potato dextrose agar (PDA) amended with cycloheximide, chlortetracycline, nystatin, streptomycin sulfate and Igepal. The CCNS cultures are incubated for 7 days at 25°C with 12-hour photoperiods. Colonies of KRL-AG2 are initially off-white in colour, producing little to no diffusible pigment in the surrounding agar medium. After several days of incubation, KRL-AG2 colonies produce green spore masses in diurnal zones of heavy sporulation, whereas other strains produce lighter colour spore masses with little diurnal variation in spore mass density.

The colony morphology of *T. harzianum* strain KRL-AG2 is influenced by such conditions as growth media and light. On PDA, the perimeters of the colonies are white to cottony in appearance, with green spores giving a pale to dark green colour to the centre of the colony. Under dense conditions, the white aerial hyphae are less extensive, giving rise to a denser spore mass.

2.1.2 Methods for Establishment of Purity of Seed Stock

Both the original mother culture and the starter cultures of *T. harzianum* strain KRL-AG2 are stored on grains of silica gel at −20°C at Cornell University, Ithaca, New York. Starter cultures are evaluated for genetic stability and contamination prior to use in production. Potential bacterial contaminants are monitored by plating dilutions of samples onto tryptic soy agar (TSA), whereas fungal contaminants are monitored using PDA. All bacterial and fungal colonies are purified and identified using traditional typing methods as well as molecular "genetic fingerprint" analysis when necessary. Potential relationship to known bacterial pathogens is determined. A bacterial load of $> 10^7$ CFU/g or the presence of a human pathogen will result in the rejection of

a starter culture, termination of the production process or discarding of the final product. A stock culture is rejected if any fungal or bacterial contamination is present.

2.1.3 Methods to Define the Content of the Microorganism in the Manufactured Material Used for the Production of Formulated Products

Depending on the end-product formulation being manufactured, from three to five samples are taken from each lot during production and tested (CFU test) using a standardized method to determine the viability of the active ingredient. The CFU test is performed to estimate the number of viable propagules of strain KRL-AG2 per unit mass of sample (CFU/g dry weight). The product guarantee is determined after the milling/de-agglomeration and blending process, and is expressed as CFU/g dry weight of product. No bioassays are performed to determine the potency of the final product against targeted seed pathogens.

2.1.4 Methods for the Determination of Relevant Impurities in the Manufactured Material

The applicant provided a brief discussion on the formation of extraneous material during the manufacturing process. Given that the end products are produced by growing the fungus on matrix media (powder or granular) under non-sterile conditions and the milling and blending processes also occur under non-sterile conditions, contamination is expected to occur. Manufacturing quality control procedures are designed to both minimize formation of unintentional ingredients and to monitor the level of unintentional ingredients in the final product. These procedures begin with the subculturing of the starter cultures and continue through the blending of the end products.

According to the applicant, the only significant unintentional ingredients in these end products are other soil-dwelling microorganisms, including other fungi (*Trichoderma*, *Penicillium*, *Aspergillus*, *Rhizopus*, yeast) and bacteria (streptomycetes), which are associated with an ingredient in the growth matrix. Contamination specifications, however, are in place to prevent end products from containing levels of microbial contaminants that might affect the product efficacy or storage stability. A human pathogen screening process is also in place. The end-use products do not contain or produce any known human or animal pathogen.

Procedures are in place to monitor the presence of microbial contaminants in the end-use products. Analysis data from five batches each of RootShield Granules and RootShield Drench WP were assessed for the presence and level of contamination by bacteria and fungi. The total bacterial count for RootShield Granules ranged from 1.0×10^5 to 1.1×10^6 CFU/g and the total count for RootShield Drench WP was an order of magnitude higher at 3.5×10^6 to 1.5×10^7 CFU/g. Fungal contamination in all batches was below 1.0×10^5 CFU/g. Contamination less than this concentration was not considered significant and not recorded. Isolated colonies of bacteria and fungi were not taxonomically identified.

Additional microbial analysis of three production batches each of T-22G Biological Plant Protectant Granules (a granular formulation similar to RootShield Granules) and T-22 Planter

Box (a wettable powder formulation similar to RootShield Drench WP) revealed the presence of bacterial and fungal contaminants. In both formulations, total mean populations of bacteria and fungi were within the same order of magnitude of 3.1×10^7 to 5.0×10^7 CFU/g. These contamination levels were equivalent to the microbial background levels found in the formulants. There was no significant difference found in mean population levels between final product and formulants. As an added precaution, cultures of the microbes detected were sent to Cornell University for identification. All were identified as common soil-inhabiting species, including *Trichoderma*, *Penicillium*, *Aspergillus*, yeast and *Streptomyces*. The common airborne fungus *Rhizopus* also was detected. In previous studies, production samples were tested by Rochester General Hospital (Rochester, New York) and found to be free of animal and human pathogens, including *Escherichia coli*, *Staphylococcus*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Yersinia* sp., *Salmonella*, *Shigella*, *Clostridium perfringens*, *Bacillus* and *Campylobacter*. No pathogens were detected in any of the five consecutive production batches tested.

No known toxic metabolites or hazardous substances are present in the RootShield Drench WP and RootShield Granules end-use products. However, *T. harzianum* is a prolific producer of secondary metabolites that include numerous types of alkyl pyrones, isonitriles, polyketides, oxygen heterocyclic compounds, diketopiperazines and terpenoids (sesquiterpenes, diterpenes), which act either as antifungal/antibacterial agents or plant growth regulators. Of the many antibacterial and antifungal metabolites produced by *T. harzianum*, only a few present a potential risk to applicators and consumers of treated food crops. Of special interest is a unique class of linear hydrophobic polypeptides called peptaibols, which are produced by most species and strains of *Trichoderma* including *T. harzianum*. Peptaibols function as antibiotics and contain a high proportion of α,α-dimethylisobutyric acid. Many peptaibols, such as the trichorzianines, trichokindins, trichorzins, trichorozins and harzianins, exhibit a broad range of bioactivities related to cell membrane perturbation. These activities include such in vitro effects as hemolysis, uncoupling of oxidative phosphorylation in rat liver mitochondria, inhibition of multiplication of different types of cells, acting as channel agonists in bullfrog cardiac myocytes and enhancing secretion of catecholamine from adrenal chromaffin cells. In a study submitted by BioWorks Inc., the MPCA's potential to produce these peptaibols was studied under conditions that favoured their production. The results of this study clearly showed that strain KRL-AG2 produced four peptaibols, namely trichorzins HA II and HA V, and harzianins HB I and HC XIII. However, no new peptaibols were isolated and no significant adverse effects were reported in Tier I acute toxicity/pathogenicity studies. Analytical methods to detect peptaibols in the end-use products are available, but their analysis will not be required in the manufacturer's quality assurance program since the likelihood of adverse toxicological effects is expected to be acceptably low and the costs associated with the analysis are considered to be too prohibitive to warrant routine monitoring.

There are no other known mycotoxins produced by *T. harzianum*, although one report in the literature attributed the production of certain trichothecene mycotoxins (i.e., trichodermin and trichodermol) to a fungus identified as *T. harzianum* but later believed to be a misidentified isolate of *Trichoderma atroviride*. *Trichoderma atroviride* is not closely related to *T. harzianum*, but is more closely related to *Trichoderma viride*, which is a known producer of these mycotoxins.

2.1.5 Methods for Demonstration of Absence of Any Human and Mammalian Pathogens

As discussed above, the quality assurance program implemented by the applicant for the production of the two RootShield end-use products requires the destruction of the batch if any human or animal pathogens are detected during the manufacturing process.

2.1.6 Methods for Determination of Storage Stability, Shelf-Life of the Microorganism

Storage stability testing was conducted on representative batches of RootShield Granules and RootShield Drench WP formulations. Final product samples were stored in the dark at 0–6°C, except for brief periods when samples were removed for testing. No other environmental factors were controlled or monitored. Fungal viability (CFU) agar plate counts were conducted every three months, but only the last test results were reported for each sample. The required minimum specifications for CFU counts were maintained in a majority of the 14–19 batches for up to 12 months of storage. Current directions for use and storage recommend users store the product at temperatures less than 5°C and use the product within 12 months. These recommendations are consistent with the product storage information.

2.2 Methods for Determination and Quantification of Residues (viable or non-viable) of the Active Microorganism and Relevant Metabolites

As noted in Section 2.1.4, four types of peptaibols were isolated from cultures of *T. harzianum* strain KRL-AG2 grown under conditions that favoured their production. Given that these four peptaibol types were also isolated from a culture of a native strain of *T. harzianum*, it is likely that crops grown in untreated soil would be exposed to these antibiotic peptides. Consequently, food from crops treated with strain KRL-AG2 would only be considered as adulterated if peptaibol residues on treated crops exceeded those of untreated crops. However, the likelihood of these peptaibol residues exceeding naturally occurring levels is considered to be low. Although the crude absolute weight of peptaibols recovered from strain KRL-AG2 was slightly greater compared to the native strain, this weight was not adjusted for dry mycelial weight and, thus, the relevance of this observation is questionable. As peptaibol production is directly affected by growth rate, and strain KRL-AG2 was selected for its potential to rapidly colonize plant roots, there is no direct evidence to suggest that strain KRL-AG2 in RootShield produces more peptaibols than native strains. Moreover, strain KRL-AG2 does not produce novel peptaibols compared to the native strain.

As no significant adverse effects were reported in Tier I acute toxicity / pathogenicity studies and there are no definitive data that suggest strain KRL-AG2 produces peptaibols above levels produced by naturally occurring isolates of *T. harzianum* in the environment, the establishment of a maximum residue limit (MRL) is not required for *T. harzianum* Rifai strain KRL-AG2. Consequently, no method(s) to quantify *T. harzianum* Rifai strain KRL-AG2 residues in food and feed are required.

There are no known international MRLs or tolerances for this MPCA and its metabolites; the United States Environmental Protection Agency (USEPA) granted an exemption from the requirement of a tolerance for strain KRL-AG2 in and on all food and feed commodities.

Analytical methods for detecting viable strain KRL-AG2 residues in animal and human body tissues involve blending of tissues and recovery on antibiotic- and fungicide-supplemented Trichoderma Selective Medium, which is specially formulated to support only strain KRL-AG2 colony growth.

3.0 Impact on Human and Animal Health

See Appendix I, Table 1, for a summary table of toxicity and infectivity test data.

3.1 Integrated Toxicity and Infectivity Summary

The registration package submitted by BioWorks Inc. in support of RootShield Technical, RootShield Drench WP and RootShield Granules, containing the fungus *T. harzianum* strain KRL-AG2 as the active ingredient, was reviewed from the viewpoint of human health and safety and was found to be sufficiently complete to permit a decision on registration. The information provided to address the characterization of the active ingredient as well as the manufacturing process and quality control adequately addressed the potential human health and safety concerns associated with *T. harzianum* strain KRL-AG2 and bacterial/fungal contaminants introduced during production. Although data were provided that showed strain KRL-AG2 could produce peptaibols under conditions favouring the production of these antibiotic peptides (see sections 2.1.4 and 2.2), the quantities produced by strain KRL-AG2 were not significantly different compared to a native strain of *T. harzianum*, and no new peptaibols were isolated.

In toxicological studies, *T. harzianum* strain KRL-AG2 did not show any signs of toxicity or pathogenicity when administered to rats via the oral or intravenous routes. The observation of enlarged spleens in treated animals following intravenous injection was considered to be a normal reaction to a high dose of foreign agent. Intratracheal instillation of the test organism showed no apparent signs of treatment-related pathogenicity. Lesions consisting of mottled or enlarged and mottled lungs were observed in the pulmonary tract of treated male and female animals. Although lesions have been known to be associated with the instillation of a large number of microorganisms, particularly fungi, directly into the lungs, the gross lesions in this study appeared to be typical of a normal immune response in healthy animals. A request to waive the actual dermal toxicity and the primary dermal irritation study requirements for RootShield Drench WP and RootShield Granules end-use products was accepted based on a reported absence of adverse effects in workers involved in the manufacture of these products, the non-toxicity and widespread commercial use of the inert formulation ingredients (formulants) as well as the low toxicity and no pathogenicity ranking of the active microorganism in acute oral, pulmonary and intravenous tests. A purified preparation of the test organism was minimally irritating to the rabbit eye; however, the RootShield end-use products contain formulants that are known to be mild ocular irritants.

Based on the absence of significant adverse effects in the Tier I maximum hazard studies, higher tier toxicity/pathogenicity studies involving subchronic and chronic testing, oncogenicity testing, mutagenicity and teratogenicity are not required for *T. harzianum* strain KRL-AG2. Furthermore, there are no known toxicological concerns associated with any of the formulants contained in RootShield Drench WP and RootShield Granules.

The microbial active ingredient, *T. harzianum* strain KRL-AG2, is not known to be a human pathogen nor an endocrine disrupter. The submitted toxicity/pathogenicity studies in the rodent indicate that the immune system is still intact and able to process and clear the active ingredient following exposure via several routes. Therefore, no adverse effects to the endocrine or immune systems are known or expected.

3.2 Reporting of Hypersensitivity Incidence

A skin sensitization study was not submitted on the microbial active ingredient, *T. harzianum* strain KRL-AG2, as hypersensitivity studies are not required by the PMRA to support the registration of microbial pest control agents. Skin-sensitizing studies are not considered substitutes for timely reports of hypersensitivity incidents subsequent to registration approval.

During product research and development activities as well as operational applications in the United States where the active ingredient has been registered since 1990, individuals have been exposed to both mycelia and spores of the MPCA. Exposure is likely to have occurred via dermal and inhalation routes. No report, or suggestion, of hypersensitivity to this fungus has been noted during this time. Nevertheless, because most microorganisms contain substances that elicit positive hypersensitivity reactions in humans, *T. harzianum* strain KRL-AG2 is considered to be a potential sensitizing agent. Consequently, the signal words "POTENTIAL SENSITIZER" are required on the principal display panels of the RootShield technical and end-use formulation labels. The use of personal protective equipment is also required to mitigate against potential dermal sensitization in occupationally exposed workers/handlers. The applicant will be expected to report any subsequent findings of hypersensitivity or other health incidents to workers, applicators or bystanders exposed to the MPCA as a condition of registration. Incident reports are to include the following details:

- a description of the MPCA and formulation;
- the frequency, duration and routes of exposure to the material;
- clinical observations; and
- any other relevant information.

3.3 Impact on Human and Animal Health Arising from Exposure to the Active Substance or to Impurities Contained in It

3.3.1 Occupational and Bystander Exposure Assessment

The PMRA does not expect that occupational exposures will pose an undue risk on the basis of the low toxicity/pathogenicity profile for *T. harzianum* strain KRL-AG2 and the assumption that

precautionary label statements will be followed in the use of RootShield Drench WP and RootShield Granules.

The potential for dermal, eye and inhalation exposure for pesticide handlers exists, with the major source of exposure to workers being generally dermal. As unbroken skin is a natural barrier to microbial invasion of the human body, dermal absorption could occur only if the skin were cut, if the microbe were a pathogen equipped with mechanisms for entry through or infection of the skin or if metabolites were produced that could be dermally absorbed. *T. harzianum* strain KRL-AG2 is not known to be a human pathogen nor is it known to produce metabolites that are dermally absorbed. Based on the demonstrated lack of adverse effects in the intravenous study, it is the PMRA's opinion that entry of absorbed *T. harzianum* strain KRL-AG2 into the body, even through cut skin, should not pose a risk to health. Furthermore, the pesticide has generated no incidents of skin problems (including hypersensitivity) in workers who have had frequent dermal exposure to strain KRL-AG2. However, as dermal exposure studies conducted on another USEPA-registered Rifai strain of *T. harzianum*, strain T-39, indicated the potential for dermal irritation and delayed contact hypersensitivity, the PMRA has imposed label restrictions and risk mitigation measures to protect populations who are likely to be primarily exposed to strain KRL-AG2 from greenhouse applications of RootShield Drench WP and RootShield Granules. Such exposure to pesticide handlers can be reduced if they wear a long-sleeved shirt, long pants, waterproof gloves, shoes and socks.

A pure powder preparation of strain KRL-AG2 was shown to be minimally irritating to the rabbit eye, but because the RootShield end-use products both contain ingredients (formulants) that are eye irritants, this concern can be adequately addressed if protective eye goggles are worn by workers.

While submitted studies on strain KRL-AG2 indicated a potential for a mild pulmonary risk, inhalation exposure is not of concern if the required dust/filter respirator is also worn by applicators and early-entry (postapplication) workers. Consequently, pesticide handlers must wear a dust/mist filtering respirator with the National Institute of Occupational Safety and Health (NIOSH) prefix –95, P-95 or R-95 to mitigate exposure. A restricted entry interval of four hours will also be required on end-product labels to protect early entry workers or other persons entering treated greenhouses.

The label does not allow applications to turf, residential or recreational areas. Because the use sites are agricultural (greenhouse), exposure to infants and children in school, residential or daycare facilities is likely to be minimal to non-existent. Consequently, the health risk to infants and children is expected to be negligible to non-existent.

4.0 Residues

4.1 Residue Summary

Even though *T. harzianum* is a ubiquitous organism found in most terrestrial environments, *Trichoderma* species are rarely reported to occur on living plants. The proposed food use pattern,

therefore, is likely to result in only low levels of dietary exposure or residues on treated food commodities at the time of harvest. Furthermore, any residues of the active microorganism are likely to be removed from treated food by washing, peeling, cooking and processing. Even if residues are not removed, dietary exposure to the microbial agent is unlikely to result in any undue hazard to consumers because no adverse effects were observed at maximum hazard dose levels in the submitted Tier I acute oral study.

The PMRA did not require subchronic and chronic dietary exposure studies because the Tier I acute oral study demonstrated a low toxicity and no pathogenicity potential for the active microorganism. Because of the low toxicity profile and low exposure potential of the MPCA, there is no concern for chronic risks posed by dietary exposure of sensitive subpopulations, such as infants and children.

Dietary exposure to secondary metabolites produced by *T. harzianum* strain KRL-AG2 is possible, even though no aerial parts of tomato and cucumber crops will be directly treated with the MPCA. Uptake by plant roots and translocation to cucumbers and tomato fruit is possible for metabolites produced by the actively growing fungus in greenhouse media, but no crop residue data were submitted for any of the secondary metabolites that may present a human health concern, specifically peptaibol antibiotics. However, given the record of safe use of products containing this active ingredient in the United States as indicated by the absence of adverse effects reports, residue levels of these metabolites are likely to be sufficiently low in the crop at the time of harvest so as not to provoke a concern for dietary exposure.

Trichoderma harzianum is not generally recognized as an aquatic microorganism. Thus, it is not expected to proliferate in aquatic habitats following incidents of direct or indirect exposure (e.g., runoff from treated greenhouses). Moreover, *T. harzianum* is not considered to be a risk to drinking water. Accordingly, drinking water is not specifically screened for *T. harzianum* as a potential indicator of microbial contamination or as a direct pathogenic contaminant. Both percolation through soil and municipal treatment of drinking water would reduce the possibility of significant transfer of residues to drinking water. Therefore, the potential of exposure and risk via drinking water is likely to be minimal to non-existent for this MPCA.

4.2 Maximum Residue Limit

As no significant adverse effects were reported in Tier I acute toxicity/pathogenicity studies and no definitive data are available that would suggest the MPCA produces secondary metabolites of potential health concern above levels produced by naturally occurring isolates of *T. harzianum* in the environment, the establishment of an MRL is not required for *T. harzianum* Rifai strain KRL-AG2 under Section 4(d) of the *Food and Drugs Act* (adulteration of food) as defined under Division 15, Section B.15.002 of the Food and Drugs Regulations.

The USEPA granted tolerance exemptions for strain KRL-AG2 and another Rifai biological control strain of *T. harzianum*, strain T-39. As well, there is no Codex MRL for any strain of *T. harzianum*.

5.0 Fate and Behaviour in the Environment

Environmental fate data (Tier II/III) were not required as significant adverse effects to most non-target organisms were not expected from the proposed use of the MPCA.

5.1 Summary of Fate and Behaviour in the Terrestrial Environment

5.1.1 Field Studies

BioWorks Inc. submitted an overwintering study that studied *T. harzianum* KRL-AG2's ability to persist and disseminate in fields sown with treated corn and soybean seeds. The crops were grown following normal agronomic practices with the exception that no conventional chemical fungicides were applied. Soil and root debris from the root zones of treated and untreated plants were collected in the following fall and spring, and samples were assayed for *T. harzianum* KRL-AG2 and other *Trichoderma* species. After the plots were assayed in the spring, untreated seeds of corn and soybean were sown in the plots and the crops were grown as the previous year. After the crops matured, these were harvested, and the roots were assayed for *T. harzianum* KRL-AG2 and other *Trichoderma* species.

Trichoderma harzianum KRL-AG2 was detected in soil collected from the treated and untreated plots in the spring and, thus, survived over the winter. Its isolation from soils collected from untreated crops suggests that the active ingredient was rapidly disseminated in soil. Surviving populations of *T. harzianum* KRL-AG2 were also shown to colonize the roots of subsequent crops to a limited extent, but had no observed effects, either beneficial or deleterious, on subsequent crops.

5.1.2 Conclusions

Trichoderma species are ubiquitous soil-dwellers, inhabiting soil, rotting wood and vegetable matter in virtually all terrestrial environments. They produce copious conidia held together in mucoid spore balls, which can be disseminated by water and by soil fauna such as insects and earthworms. With respect to its abundance relative to other species of *Trichoderma*, *T. harzianum* has been characterized as more characteristic of warm climates; however, it is evident from the field study and published literature that cold-tolerant strains do exist. It is also evident that *T. harzianum* KRL-AG2 will likely disseminate and persist in the Canadian environment following its release. Adverse effects, however, are not expected as *T. harzianum* is a saprophyte, with the exception that it can attack other fungi. Furthermore, *T. harzianum* KRL-AG2 has been used in the United States for a number of years with no reports of adverse environmental effects.

6.0 Effects on Non-Target Species

See Appendix I, Table 2 and Table 3, for summaries.

6.1 Effects on Terrestrial Organisms

Acceptable waiver rationales were submitted to address all terrestrial environmental toxicology requirements. These rationales were based on minimal exposure due to the products' proposed use patterns, the ubiquitous nature of *T. harzianum*, lack of reported adverse effects in the literature. Granted some aggressive biotypes of *T. harzianum* were identified as the causal agent of "green mould disease" in mushrooms, but the risk to the commercial mushroom industry is considered to be low if the products are not used on mushroom farms and if treated plant materials are not used as substrate by mushroom growers.

An acute avian oral study was submitted, but it was not accepted. This data requirement, however, was not considered necessary to assess the risks of *T. harzianum* KRL-AG2 as the potential for exposure via the oral route is expected to be minimal when applied in the greenhouse.

The formulation ingredients identified in Rootshield Drench WP and Rootshield Granules do not pose a risk to non-target terrestrial organisms when used at the proposed concentrations and application rates.

6.2 Effects on Aquatic Organisms

Acceptable waiver rationales were submitted to address all aquatic environmental toxicology requirements. These rationales were based on minimal exposure due to the products' proposed use patterns, the ubiquitous nature of *T. harzianum*, lack of reported adverse effects in the literature and the inability of *T. harzianum* to become established in unpolluted aquatic environments.

The formulation ingredients identified in Rootshield Drench WP and Rootshield Granules do not pose a risk to aquatic organisms when used at the proposed concentrations and application rates.

6.3 Risk Characterization

Trichoderma species are ubiquitous soil-dwellers, inhabiting soil, rotting wood and vegetable matter in virtually all terrestrial environments. The field study submitted by the applicant demonstrated that *T. harzianum* KRL-AG2 disseminated rapidly in the environment and that it persisted in the environment following its release. Those findings were consistent with published literature; thus, the active ingredient, a fusion product of two auxotrophic strains of *Trichoderma* species, behaved as expected. From the viewpoint of environmental protection, some of the information and data in the published literature raised some concerns. Its mode of action presented potential non-target effects against beneficial soil microorganisms such as mycorrhizal fungi. Furthermore, the plant-regulating metabolites and cellulolytic enzymes produced by this species could negatively affect non-target plants. Many of those environmental concerns, however, are alleviated by the proposed use patterns for both Rootshield Drench WP and Rootshield Granules WP and by the absence of documented adverse effects in the published literature.

Trichoderma harzianum KRL-AG2 will only be applied on greenhouse food and non-food crops; hence, the potential for exposure is greatly reduced. In addition, relatively few cases of adverse effects have been reported. Those references reporting adverse effects dealt mostly with the identification of *T. harzianum* as the causal agent of "green mould disease". Although the active ingredient's potential to cause "green mould disease" is unknown, Rootshield Drench WP and Rootshield Granules will not be used in mushroom farms. Furthermore, a mitigative statement will prevent greenhouse operators from distributing the treated plant matter to mushroom growers for use as substrate. The remaining few references dealt with the issue of plant growth regulating metabolites. Phytotoxicity was reported; however, those studies used concentrations that were much greater than those expected in natural settings. Phytotoxicity occurrences are unlikely in non-target plants as *T. harzianum* will be applied in greenhouses.

Based largely on published literature, waivers were submitted for the environmental toxicology requirements. Non-target organisms will face minimal increased exposure to *T. harzianum* as a result of the use of Rootshield Drench WP and Rootshield Granules. The formulants in the end-use products do not pose an environmental risk when used at the proposed concentrations and application rates. Consequently, Rootshield Drench WP and Rootshield Granules are expected to pose little environmental risk when used in accordance with the label directions. The following label statement, however, will be required on the secondary panel of the label under the section **ENVIRONMENTAL PRECAUTIONS**:

> "The treated plant material must not be used as substrate for mushroom farms."

7.0 Efficacy

7.1 Effectiveness on Selected Diseases

RootShield products, RootShield Drench WP and RootShield Granules, contain 1.15% (1×10^7 CFU/g) *T. harzianum* Rifai strain KRL-AG2. These biological fungicides are proposed for the suppression of root diseases caused by *Pythium*, *Rhizoctonia* and *Fusarium* on tomato, cucumber and ornamental crops grown in greenhouses. RootShield can be used alone or in conjunction with certain chemical fungicides. RootShield is claimed to provide prolonged protection for the root system of crops extending from stand establishment through to harvest.

The proposed labels indicate that RootShield Drench WP should be suspended in sufficient water to guarantee good wetting of the culture medium or soil, but runoff and excess drainage should be avoided. RootShield Drench WP is to be applied at 6 grams product per square metre of cultivated area (suspended in 2.5–5 litres of water) immediately after sowing or planting cuttings (non-rooted or rooted) for propagation, at 15 grams product per square metre of cultivated area (suspended in 10 litres of water) or at 150 grams per 1000 pots (suspended in 100 litres of water) for cultivation. Treatments of RootShield Drench WP can be repeated every 10–12 weeks at the full label rate or after re-potting plants into a larger container using half of the dose. Ornamental bulbs are to be dipped in suspension of 120 g/L RootShield Drench WP prior to planting. RootShield Granules will be incorporated into greenhouse potting mix or soil at a rate of 750 g product per cubic metre of (loose) mix.

A total of 28 efficacy trials on greenhouse ornamentals, tomatoes, cucumbers, onions, eggplants and peppers were reviewed. In most cases, potting mix was treated with RootShield Drench WP or RootShield Granules as proposed and then infested with the target pathogens *Pythium* or *Rhizoctonia*. In three greenhouse trials efficacy of RootShield Drench WP was determined for the control of *Pythium*, *Fusarium* and *Rhizoctonia* on tomato and cucumber grown on rockwool using hydroponic culture. The interval between treatment and inoculation typically varied from 0–10 days. For *Fusarium* tests, seedlings were grown in the greenhouse in presence of RootShield products and then transplanted at infested field sites. Plants were assessed for signs of disease such as dead plants or root rot and for growth factors such as plant height, fresh weight and dry weight of tops or roots. The marketable quality of flowering plants as well as quantity and quality of vegetables were also assessed. Trials were conducted using 60–150 g RootShield Drench WP in 100 L water or 560–600 g RootShield Granules per cubic metre of potting mix.

Researchers typically considered RootShield to have an overall positive effect on plant health although this was not always evident in the measured variables. The level of demonstrated benefit in these trials is more appropriately termed *suppression* than control. In *Rhizoctonia* trials, RootShield Drench WP resulted in greater plant survival (8–44%) compared to the inoculated check, as well as increased plant weight and slight increase in root/shoot grade among ornamentals. In *Pythium* trials, RootShield increased root dry weights and plant fresh weights of certain ornamental varieties and resulted in improved yield in tomatoes. *Fusarium* trials showed a 25–57% reduction in crown and root rot and increased yield in field tomatoes treated with RootShield prior to transplant. One or two drench applications of RootShield at 75–150 g/100L to rockwool blocks significantly suppressed root diseases caused by *Pythium* and *Fusarium* in hydroponically grown tomato and cucumber crops. In addition, when RootShield alone was added to the rockwool blocks, stimulation of root growth was observed. A trial with various treatment methods showed improved yield of onion transplants dipped in a RootShield Drench WP suspension.

The submitted data on RootShield Drench WP and RootShield Granules suggest that these products have potential to suppress diseases due to *Pythium, Rhizoctonia* and *Fusarium*. These data also suggest these products may have a positive effect on plant growth, especially roots, even in the absence of target pathogens. The efficacy results of these trials were variable; in some cases, RootShield products provided significant suppression of diseases, in others, they were ineffective against the pathogens. This variability is typical of microbial products due to their interaction with other organisms and the soil environment. Nonetheless, there were sufficient positive data to support claims of suppression of these three pathogens on greenhouse tomatoes, cucumbers and ornamentals.

Several factors appear to favour RootShield and improve efficacy. One of these factors is timing of application: researchers noted that the product needs to be introduced well before the pathogen. In practice, this means treating potting mix with RootShield 7–14 days prior to using it for seeding or transplanting, or drenching the seedlings as soon as possible to favour colonization of the roots prior to infection by the pathogens.

Based on submitted data, RootShield Drench WP and RootShield Granules are supported for suppression of *Rhizoctonia, Pythium* and *Fusarium* on tomatoes and cucumbers as well as for *Rhizoctonia* and *Pythium* on ornamentals. Only drench and bulb dip (120 g/L) applications using RootShield Drench WP are supported for suppression of *Fusarium* on ornamentals.

Use of RootShield Drench WP as a dry product (undiluted powder) for ornamental cuttings or bare root transplants was not assessed and is not accepted. Although no efficacy trials were conducted with RootShield Granules at 750 g/m^3 of potting-mix, based on the results of the trials conducted at 560–600 g/m^3 of potting-mix, the proposed rate 750 g/m^3 of potting-mix is supported. The 750 g/m^3 rate is more consistent with the USEPA-approved label. In addition, most growers find it easier to blend 750 g/m^3 of potting mix or soil.

Additional efficacy data demonstrated the efficacy of RootShield Drench WP at 75–150 g/L water against *Pythium* and *Fusarium* diseases on tomatoes and cucumbers grown hydroponically on rockwool. Specific use directions have been added to the label for hydroponic production of greenhouse vegetables.

7.2 Phytotoxicity/Pathogenicity to Target Plants or to Target Plant Products

Phytotoxicity caused by RootShield products in the absence of soil pathogens was not observed. All ornamental varieties cannot be assessed in research trials; therefore, it is recommended that RootShield be tested on a small sample of each new variety prior to commercial scale use.

7.3 Compatibility with Current Management Practices Including Integrated Pest Management

No data were submitted to demonstrate the compatibility of RootShield products with chemical fungicides and insecticides commonly used in greenhouses. Caution must be taken when RootShield is used in alternation with a chemical fungicide. RootShield application methods require further development for use in integrated pest management (IPM) systems, but it is expected that RootShield will contribute to root disease management without any adverse effect on other IPM tools such as beneficial insects or sanitation practices.

7.4 Contribution to Risk Reduction

RootShield biological products contribute to suppression and management of plant diseases that might otherwise require frequent application of chemical fungicides for adequate control. This will reduce fungicide use in greenhouses, with consequent reduction in worker, food and environmental exposure.

7.5 Information on the Occurrence or Possible Occurrence of the Development of Resistance

The microbial active ingredient in RootShield products may employ either one or combination of the following mechanisms for the biological suppression of plant pathogens: mycoparasitism,

toxin production, rhizosphere competence and competition, and production of pathogen suppressive enzymes. It generally colonizes the entire plant root system and protects roots from diseases. It is not expected to be prone to resistance in target pathogens due to its broad mode of action.

7.6 Conclusions

RootShield Drench WP and RootShield Granules are accepted for suppression of *Pythium*, *Rhizoctonia* and *Fusarium* on greenhouse tomatoes, cucumbers and ornamentals. Acceptable label rates are 6 g/m^2 RootShield Drench WP in 2.5–5 L water (propagation stage) and 150 g/m^2 RootShield Drench WP in 100 L water (cultivation stage), or 750 g RootShield Granules per cubic metre of potting mix. The acceptable label rate for drench or bulb dip is 120 g /L of RootShield Drench WP. The products may be applied in suspension as a drench to potting mix, soil, planting beds or other media, rockwool in hydroponic culture or incorporated as a granular into potting media.

RootShield products are expected to contribute significantly to root disease management in greenhouse crop and ornamental production.

8.0 Toxic Substances Management Policy Considerations

During the review of RootShield Drench WP and RootShield Granules, the PMRA has taken into account the federal Toxic Substances Management Policy[1] (TSMP) and has followed its Regulatory Directive DIR99-03[2]. It has been determined that these products do not meet TSMP Track 1 criteria because the active ingredient is a biological organism; therefore, it is not subject to the criteria used to define persistence, bioaccumulation and toxicity properties of chemical control products. Furthermore, the technical grade active ingredient does not contain any byproducts or microcontaminants that meet the TSMP Track 1 criteria. Impurities of toxicological concern are not expected to be present in the raw materials nor are they expected to be generated in sufficient quantities during the manufacturing process to present a risk to human health and safety. Also, there are no formulants of toxicological concern present in the RootShield Drench WP and RootShield Granules end-use formulations.

9.0 Proposed Regulatory Decision

The PMRA has carried out an assessment of available information in accordance with the Pest Control Products Regulations and has found it sufficient to allow a determination of the safety,

---

1 The federal Toxic Substances Management Policy is available through Environment Canada's website at www.ec.gc.ca/toxics

2 Regulatory Directive DIR99-03, *The Pest Management Regulatory Agency's Strategy for Implementing the Toxic Substances Management Policy*, is available through the Pest Management Information Service. Phone: 1-800-267-6315 within Canada or 613-736-3799 outside Canada (long distance charges apply); fax: 613-736-3798; e-mail: pmra_infoserv@hc-sc.gc.ca; or through our website at www.pmra-arla.gc.ca

merit and value of the technical active ingredient RootShield Technical Biological Fungicide and end-use products RootShield Drench Biological Fungicide Wettable Powder and RootShield Granules Biological Fungicide. The Agency has concluded that the use of the microorganism *T. harzianum* Rifai strain KRL-AG2 in the technical grade active ingredient RootShield Technical Biological Fungicide and RootShield end-use products in accordance with the label has merit and value consistent with the Pest Control Products Regulations and does not entail an unacceptable risk of harm. Therefore, based on the considerations outlined above, the use of the microbial *T. harzianum* Rifai strain KRL-AG2 and RootShield end-use products for suppression of root pathogens in greenhouse tomatoes, cucumbers and ornamentals are proposed for full registration pursuant the Pest Control Products Regulations.

The PMRA will accept written comments on this proposal up to 45 days from the date of publication of this document to allow interested parties an opportunity to provide input into the proposed registration decision for these products.

List of Abbreviations

a.i.	active ingredient
bw	body weight
CFU	colony forming unit
CCNS	cycloheximide chlortetracycline nystatin streptomycin sulfate in potato dextrose agar
DNA	deoxyribonucleic acid
dw	dry weight
g	gram
IPM	integrated pest management
kg	kilogram
LD_{50}	lethal dose for 50% of the population
LOEL	lowest observable effect level
m	meter
mg	milligram
MIS	maximum irritation score
MPCA	microbial pest control agent
MRL	maximum residue limit
NIOSH	National Institute of Occupational Safety and Health
NOAEL	no observed adverse effect level
NOEL	no observed effect level
PDA	potato dextrose agar
PMRA	Pest Management Regulatory Agency
TSA	tryptic soy agar
TSMP	Toxic Substances Management Policy
USEPA	United States Environmental Protection Agency
UV	ultraviolet

Appendix I Summary Tables

Table 1 Summary of Toxicity and Pathogenicity Studies with RootShield Biological Fungicide (*Trichoderma harzianum* strain KRL-AG2)

STUDY	SPECIES/STRAIN AND DOSES	LD_{50}, NOEL/NOAEL and LOEL	TARGET ORGAN / SIGNIFICANT EFFECTS / COMMENTS
ACUTE STUDIES			
Oral	Rat—Sprague-Dawley, 13/sex, approx. 10^8 CFU/animal	$LD_{50} > 1.0 \times 10^8$ CFU/animal	No effect on body-weight gain and no clinical signs of treatment-related toxicity, infectivity or pathogenicity. No mortalities. Agent cleared from the gastrointestinal tract within 2 days of dosing and was not detected in the urine, blood or organs at any time. No significant findings observed at necropsy. **LOW TOXICITY AND NO PATHOGENICITY.**
Pulmonary	Rat—Sprague-Dawley, 15/sex, approx. 10^5 CFU/animal	$LD_{50} > 1.0 \times 10^5$ CFU/animal	No effect on body-weight gain and no clinical signs of toxicity. No mortalities. Necropsy revealed mottled lungs in 11 treated male and female rats, and enlargement of the lungs in 6 treated rats of both sexes. Lesions were typical of a normal immune response in healthy animals to the instillation of a high dose of foreign (antigenic) material. Early clearance of agent observed from body fluids and other organs, and clearance from the lungs by study termination at day 21. **LOW TOXICITY AND NO PATHOGENICITY.**
Injection	Rat—Sprague-Dawley, 15/sex, approx. 10^7 CFU/animal	$LD_{50} > 1.0 \times 10^7$ CFU/animal	No effect on body-weight gain and no apparent signs of treatment-related toxicity or pathogenicity. No mortalities. Treatment-related effect was limited to an enlargement of the spleen in male and female animals, which was considered to be a normal reaction to a microbial infection. Following injection, the test microbe was predominantly found in the liver, lungs, spleen, kidney and blood. Enlarged spleens were observed in 9 rats. A drastic reduction in number of test organisms and a distinct pattern of clearance of the agent were demonstrated by post-treatment day 21. **LOW TOXICITY AND NO PATHOGENICITY.**

STUDY	SPECIES/STRAIN AND DOSES	LD_{50}, NOEL/NOAEL and LOEL	TARGET ORGAN / SIGNIFICANT EFFECTS / COMMENTS
ACUTE STUDIES			
Dermal Toxicity and Irritation	Waiver rationale submitted in lieu of data	Not applicable	A waiver in lieu of actual testing was accepted for RootShield Drench WP and RootShield Granules end-use products based on an absence of adverse effects reports for workers involved in the manufacture and use of the end-use products in the United States; the non-toxic nature and widespread commercial use of the inert formulation ingredients (formulants); and the low toxicity and no pathogenicity ranking of the active microorganism in acute oral, pulmonary and intravenous tests. As a precautionary measure, all greenhouse workers who will be exposed to these products during mixing/loading, application and postapplication activities will be required to wear appropriate personal protective equipment (a long-sleeved shirt, long pants, shoes plus socks and waterproof gloves) to minimize dermal contact.
Eye Irritation	Rabbit—New Zealand white, 6 males, 0.1 g of technical powder (equivalent to 10^8 CFU/animal)	MIS = 2.3/110 (after 1 hour)	Slight redness in the conjunctivae was observed in all treated eyes at the 1-hour observation interval. All eyes appeared clinically normal by 72 hours and no other signs of irritation were observed during the 7-day observation period. The technical powder is minimally irritating. However, certain inert formulation ingredients (formulants) in RootShield end-use products are known to be mild ocular irritants. Protective eyewear (goggles) is recommended for applicators to reduce risk of contact. **TECHNICAL GRADE ACTIVE INGREDIENT—MINIMALLY IRRITATING** **END-USE PRODUCT—MILDLY IRRITATING**

Table 2 Summary of Effects on Terrestrial Organisms

Organism	Exposure	Test Substance	Endpoint Value / Comments
Birds: Bobwhite Quail	Acute Oral	Conidia of *T. harzianum* KRL-AG2	30-day $LD_{50} > 4 \times 10^9$ CFU/kg bw (or 11 110 mg a.i./kg bw) No signs of toxicity or pathogenicity. No mortalities. When compared to controls, there were no apparent effects on body weight or feed consumption. At necropsy, one bird in the treated group was noted with enlarged adrenal glands, but this observation was not considered treatment related as the adrenal glands in the other 41 treated birds appeared normal. **SUPPLEMENTAL** Insufficient information and data were provided to determine if the dose received by the birds was viable. A replacement study is not required based on the absence of adverse effects in the published literature and the low potential for avian exposure from greenhouse use.
Birds	Pulmonary/ Inhalation/ Injection	Waiver rationale submitted in lieu of data	The request for a waiver was supported with the results of the acute bobwhite quail oral (see above) and rat pulmonary studies (see Table 1). Although no treatment-related adverse effects were noted, those results were not sufficient to support the request due to difficulties in extrapolating the results of oral studies to pulmonary studies and of mammals to birds. Rather, the request was supported with literature and the proposed use-patterns for both formulations (i.e., greenhouse only). Based on the absence of adverse effects in the published literature and the low potential for avian exposure, this request for a waiver was **ACCEPTED**.

Organism	Exposure	Test Substance	Endpoint Value / Comments
Wild Mammals	Acute	Waiver rationale submitted in lieu of data	The waiver request was supported by references to toxicological studies that were previously submitted to the PMRA for the registration of RootShield Granules under the Import for Manufacturing and Export Program. As indicated in Section 3.0, *T. harzianum* KRL-AG2 did not show any signs of toxicity or pathogenicity when administered to rats via the oral or intravenous routes. Enlarged spleens were noted in treated animals following intravenous injection; however, this observation was considered to be a normal immunological reaction to a foreign particulate. Intratracheal instillation of the test organism showed no apparent signs of treatment-related pathogenicity. Furthermore, *T. harzianum* KRL-AG2 was found to be minimally irritating to the eyes of the rabbit. Based on the absence of significant adverse effects, this request for a waiver was **ACCEPTED**.
Terrestrial Arthropods	Acute	Waiver rationale submitted in lieu of data	The waiver request was supported with published literature. There are no references in which *T. harzianum* caused infection or any other impact on insects or other invertebrates. In one published article, no adverse effects were reported for bees treated with *T. harzianum* T-39. In another, honey bees and bumble bees were used to disseminate *T. harzianum* KRL-AG2 without ill effects. Other literature reported that insects, especially mites, consumed the hyphae of *Trichoderma* species. Based on the absence of adverse effects in the published literature, this request for a waiver was **ACCEPTED**.
Non-arthropod Invertebrates	Acute	Waiver rationale submitted in lieu of data	The waiver request was supported with literature. There are no references in which *T. harzianum* caused infection or any other impact on insects or other invertebrates, including non-arthropods. In one published article, earthworms were fed a diet that was infested with *T. harzianum* T3a without ill effects. Based on the absence of adverse effects in the published literature, this request for a waiver was **ACCEPTED**.

Organism	Exposure	Test Substance	Endpoint Value / Comments
Microorganisms	Acute	Waiver rationale submitted in lieu of data	The waiver request was supported with published literature. Aggressive strains of *T. harzianum* were identified in the published literature as the cause of "green mould disease". Those strains were grouped into two biotypes, namely TH2 and TH4, according to their ribosomal gene sequences. Studies done on the sequences of several biological control strains of *T. harzianum* showed that they belonged to a third biotype, namely TH1. The biotype of *T. harzianum* KRL-AG2 was not specified in the application; however, this information was not considered essential for its review as it will not be used in the commercial mushroom industry. As there are no other reports of adverse effects in the published literature, this request for a waiver was **ACCEPTED** provided that a mitigative label statement is placed on the label preventing greenhouse operators from distributing treated plant material to mushroom growers as substrate.
Terrestrial Plants	Acute	Waiver rationale submitted in lieu of data	The waiver request was supported with literature. Even though *T. harzianum* is a ubiquitous organism found in most terrestrial environments, *Trichoderma* species are rarely reported to occur on living plants. Furthermore, its ability to attack living wood or plants is considered weak despite its ability to produce potent enzymes and secondary metabolites with plant growth regulating activities. Although it is possible that those concentrations of enzymes and metabolites may increase above phytotoxic levels following direct inoculation to non-target plants, such occurrences are not expected to occur from the proposed use of the end-use formulations in greenhouses. This request for a waiver was **ACCEPTED**.

Table 3 Summary of Effects on Aquatic Organisms

Organism	Exposure	Test Substance	Endpoint Value / Comments
Fish	Acute	Waiver rationale submitted in lieu of data	The waiver request was supported with a brief literature review. The review described the natural occurrences of species of *Trichoderma*. Although ubiquitous in most terrestrial environments, few references have cited instances of recovery of any species of *Trichoderma* from aqueous environments. All but one of those occurrences involved polluted waters. One reference reported the isolation of *T. harzianum* from a marine sponge. Based on *T. harzianum*'s apparent inability to establish itself in unpolluted waters and on the absence of adverse effects to fish in the published literature, this request for a waiver was **ACCEPTED**.
Arthropods	Acute	Waiver rationale submitted in lieu of data	The waiver request was supported with published literature. As noted above, *Trichoderma* species are rarely isolated from aqueous environments. Few references have reported their occurrence and only one of those occurrences involved unpolluted waters. This reference reported the isolation of *T. harzianum* from a marine sponge. Based on *T. harzianum*'s apparent inability to establish itself in unpolluted waters and on the absence of adverse effects to arthropods in the published literature, this request for a waiver was **ACCEPTED**.
Plants	Acute	Waiver rationale submitted in lieu of data	The waiver request was supported with literature. As previously noted, *Trichoderma* species are rarely reported to occur on living plants and in unpolluted aqueous environments. Given that no adverse effects to aquatic plants were reported in the published literature, this request for a waiver was **ACCEPTED**.